

SITES OF OXYGEN UPTAKE IN OCHETOSTOMA
ERYTHROGRAMMON LEUCKART & RUEPPELL
(ECHIUROIDEA)

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In the echiuroid *Urechis caupo* inhalations and exhalations of sea water by the muscular cloacal chamber during respiration occurred through the anus (Fisher and MacGinitie, 1928b). These authors pointed out that the peristaltic movements passing along the trunk of this worm not only renewed the water in its burrow but also moved that in the respiratory chamber of the gut. Redfield and Florkin (1931) observed that in *Urechis* the oxygen in the water enclosed within the burrow and in the blood was insufficient to maintain the normal metabolic rate for the duration of the low tide, during which the hemoglobin of the blood might be expected to transport an adequate supply of oxygen to the organs of the body. Hall (1931) found that the oxygen consumption of *Urechis caupo* was comparable to that of related forms.

Ochetostoma erythrogrammon in many poorly drained beaches in the tropics also feeds during low tide by protruding its proboscis outside the burrow. The reduced availability of oxygen in the burrow at ebb tide, the small diameter of the hindgut, and the irregular, infrequent and small outflows from the anus of specimens in burrows built along the glass wall of the aquarium in the laboratory suggest that the anus may not be the sole organ of respiration.

In the present study the oxygen uptake of entire specimens and of parts of the body of *Ochetostoma erythrogrammon* was determined.

MATERIALS AND METHODS

Specimens of *Ochetostoma erythrogrammon* from the intertidal muddy sand of the west coast of Singapore Island, where feeding also occurs during ebb tide, were starved for 3–5 days to allow the faecal pellets to be completely voided, and their oxygen consumption was determined in a closed bottle. As a precaution against excessive peristaltic movements each specimen was confined in a cylindrical bag 1 cm. in diameter and 6–8 cm. long, to which it was acclimatized for one day. This bag of nylon netting of 81 meshes per sq. cm. was slipped, together with the enclosed specimen, into a bottle of about 175-milliliter capacity, the actual volume of which was previously determined.

The natural sea water used was filtered into a jar with a capacity of 13 liters and thoroughly aerated before it was covered with a thick layer of oil and siphoned into individual bottles containing the experimental animals. To ensure that the water siphoned into the bottles was not in contact with air, the water in the bottle was retained only after an amount of water equivalent to twice the volume of the bottle had passed through. The experiments were carried out at 19.3, 19.5, 19.9

and $20.0^{\circ}\text{C.} \pm 0.1^{\circ}\text{C.}$ Each bottle containing the experimental animal was turned at half-hourly intervals to ensure thorough mixing.

To prevent cloacal respiration a nylon rod of suitable diameter was inserted into the cloaca via the anus and secured by ligating the posterior tip of the trunk around it. This treatment did not seem to adversely affect the specimens even after more than 9 hours of anal blockade, since they survived when the rod was released by cutting away the ligature.

The proboscis was easily detached by gently squeezing with a pair of fine forceps its attenuated junction with the trunk. Autotomy of the proboscis occurs in nature and contraction of the circular muscle of the trunk at this junction prevents bleeding. The detached proboscis continues to move for several days with its cilia still beating. Its oxygen uptake was determined immediately after its separation from the trunk.

The oxygen content was determined by the modification of Fox and Wingfield (1937) of the Winkler method using phosphoric acid. A blank control was run at the same temperature with every batch of water to find out the amount of oxygen consumed by microorganisms present in the sample of filtered water. This amount was very small for the duration of the experiments and was deducted from the amount of oxygen consumed by the specimens. The fixed tissues of *Ochetostoma* comprised the proboscis, body wall with attached nephridia and the gut wall drained of its contents. Wet weight refers to their weight after blotting with filter paper, and dry weight, after drying for 24 hours at 100°C. in an oven. The coelomic corpuscles were not included.

To record the peristaltic movements of the trunk the apparatus used was based on the same principle as the one devised by Wells (1951) but with the following modifications to suit the weak movements of *Ochetostoma*, namely the use of (1) a light rubber bung for float, (2) a weak spring attached to a lever with writing point to counterbalance the float and (3) a plastic U-tube of about 8–10 mm. diameter to exactly fit the trunk diameter of the worm.

To record the quantity of water pumped by the worm the inlet end of the plastic U-tube of an apparatus based on the one used by Hall (1931) for *Urechis* had to be submerged below the surface of the water before any water could be pumped out by the weak peristaltic movements of *Ochetostoma*.

RESULTS AND DISCUSSION

The movements of *Ochetostoma* likely to influence the oxygen consumption considerably are peristaltic and antiperistaltic movements of the trunk and the movements of the proboscis. *Ochetostoma erythrogrammon*, the biggest specimen of which barely weighs 10 gm., is a small echiuroid compared with *Urechis*. The peristaltic movements of the trunk, which is less muscular than that of *Urechis*, were weak and did not displace a large enough volume of water. Only 0–25 cc. was pumped irregularly over a period of three hours; this is less than the quantity pumped by *Urechis* in one minute (Hall, 1931). In *Ochetostoma* reared in aquaria in the laboratory peristaltic waves at the rate of four per minute may pass through the trunk. Each peristaltic movement causes a stream of water to issue from the opening of the burrow facing the posterior end of the body. These peristaltic movements serve to renew the water of the burrow for respiratory and feeding purposes.

The tracing in Figure 1A shows a spell of regular peristalsis at the rate of about three per minute, while Figure 1B shows some irregular peristaltic movements occurring in another specimen. A series of peristalses is usually succeeded by a rest period of variable duration.

Hall (1931) showed that the oxygen consumption of *Urechis* in U-tubes bore no consistent relation to oxygen partial pressure at least over the range of 138.2 to 93.3 mm. Hg. The range of oxygen tension encountered by *Ochetostoma* in nature must be considerable from flood tide to ebb tide. Under experimental conditions the range of 5.08 to 3.20 cc. oxygen per liter at the onset of the experiments is within the usual range encountered by the animals and the experiments were continued

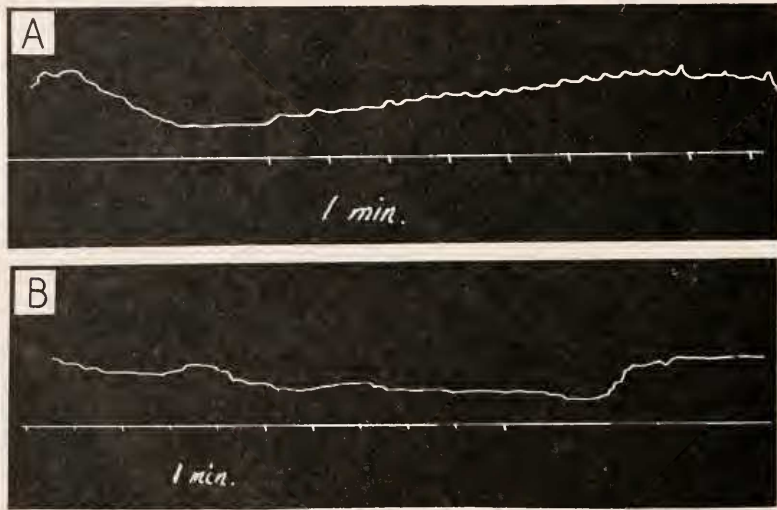


FIGURE 1. Record of peristaltic movements of *Ochetostoma erythrogrammon*. Read from left to right. Time: one division per minute. Each convex (upward) part of the curve represents the passage of a peristaltic wave along the trunk. A. Record of a specimen with regular peristaltic movements. B. Record of another specimen with peristaltic movements occurring at irregular intervals.

until the oxygen consumed amounted to about 25% of the original amount except in specimen 12 where the experiment was continued until the oxygen content dropped to 1.1 cc./liter. In this case the consumption was not far below the mean, indicating that a fall in oxygen tension did not materially affect oxygen uptake.

In Table I the blocked anus of specimens 1-8 was released at the start of the second period. In specimens 9-12 the anus was blocked at the start of the second period. With the exception of specimens 8 and 9, the rate of oxygen uptake was greater during the second period irrespective of the state of the anus and the oxygen tension. Since Hall (1931) showed that the oxygen consumption in *Urechis caupo* almost doubled with increased activity, presumably there was a tendency towards increased activity during the second period, making it difficult to assess the true effects of blocking the anus. Although the peristaltic movements of *O. erythrogrammon* were subdued by confinement in a nylon bag, it was not possible to

TABLE I

Rate of oxygen consumption of Ochotostoma erythrogrammon

No.	Specimen		Duration per period	Animal with blocked anus			Normal animal		
	Weight in grams			Initial O ₂ content	Oxygen uptake based on		Initial O ₂ content	Oxygen uptake based on	
	wet	dry			wet weight	dry weight		wet weight	dry weight
				Period I			Period II		
1	0.5585	0.0845	2	4.32	0.0710	0.4691	4.00	0.0715	0.4725
2	0.9024	0.1440	2	4.32	0.0580	0.3633	4.00	0.0635	0.3802
3	0.6577	0.0947	4	4.84	0.0512	0.3556	4.82	0.0633	0.4395
4	0.5041	0.0939	4	4.84	0.0761	0.4084	4.80	0.0962	0.5163
5	0.8646	0.1437	4	4.84	0.0571	0.3436	4.80	0.0660	0.3969
6	0.6347	0.1158	4	4.84	0.0618	0.3388	4.74	0.0827	0.4531
7	0.5955	0.1025	4	4.84	0.0826	0.4808	4.74	0.1030	0.5993
8	0.5928	0.0772	4	4.84	0.0613	0.4704	4.74	0.0513	0.3943
				Period II			Period I		
9	1.5515	0.2497	4	5.08	0.0358	0.2225	4.49	0.0369	0.2295
10	0.6624	0.1085	4	5.08	0.1330	0.8120	4.49	0.1021	0.6231
11	0.7096	0.1145	4	4.85	0.1134	0.7031	4.49	0.0539	0.3343
12	0.9370	0.1621	9½, 8½	4.64	0.0697	0.4031	4.00	0.0542	0.3135
Mean uptake:					0.07258	0.4476		0.07038	0.4294

ensure that the activity was of equal intensity between the various experimental periods.

In cases where the oxygen uptake fell after blocking of the anus, the fall was small, however, indicating that the cloaca and hindgut in *O. erythrogrammon* are of no respiratory significance in contrast to *Urechis caupo* (Fisher and MacGinitie, 1928b; Redfield and Florkin, 1931).

Hall (1931) pointed out the large amount of blood present in *Urechis* and its inclusion in the weight of tissues in the calculation for the rate of oxygen uptake would give a low value. Similarly, in 10 specimens of *O. erythrogrammon* the blood and gut fluid averaged 82% of total weight of the animal against 35% for *Urechis caupo* (Hall, 1931) and were not included in the calculation of oxygen consumption.

Table II shows that both trunk and proboscis, when separated from each other, consumed oxygen. With the exception of specimens 1 and 14, the trunk consumed more oxygen than the entire animal during the first experimental period, thus demonstrating the variability of oxygen uptake and establishing the trunk as the chief respiratory organ in this species. The higher uptake of the proboscis-less trunk was probably due to increased activity of the trunk after the loss of the proboscis. Hence in addition to its role in pumping and renewing the water in the burrow for respiratory and feeding purposes, the trunk of *O. erythrogrammon* also serves as a respiratory surface for oxygen uptake, for which it is well suited be-

TABLE II

Rate of oxygen consumption of the trunk and proboscis of Ochetostoma erythrogrammon in cc./gm./hr.

Specimen No.	Trunk wet weight	Proboscis wet weight	Duration of period	Period I: Normal animal			Period II: Trunk separated from proboscis				
				Initial O ₂ content	Oxygen uptake based on		Initial O ₂ content	Trunk O ₂ uptake based on		Proboscis O ₂ uptake based on	
					wet weight	dry weight		wet weight	dry weight	wet weight	dry weight
				gm.	gm.	hrs.	cc./liter	cc.	cc.	cc./liter	cc.
1	0.3721	0.1864	2	4.00	0.0715	0.4725	3.40	0.0976	0.5430	0.0458	0.4853
2	0.5678	0.3346	2	4.00	0.0635	0.3802	3.40	0.1212	0.6226	0.0700	0.6996
3	0.3963	0.2614	2	3.32	0.0544	0.3781	3.20	0.1007	0.5443	0.0365	0.4455
4	0.4515	0.0526	2	3.32	0.0786	0.4222	3.20	0.1198	0.6152	0.1131	0.9916
5	0.6651	0.1995	2	3.32	0.0341	0.2054	3.20	0.0576	0.3015	0.0158	0.1910
6	0.5105	0.1242	2	3.32	0.0669	0.3666	3.20	0.0886	0.4416	0.0843	0.7817
13	0.4454	0.2102	2	4.32	0.0719	0.4457	4.00	0.1313	0.6667	0.0545	0.6367
14	0.5174	0.2741	3	3.90	0.1302	0.8280	4.89	0.1513	0.8062	0.0463	0.4627
15	0.8285	0.3020	3	3.49	0.0345	0.2400	5.08	0.0507	0.3073	0.0453	0.5240
Mean uptake:					0.0672	0.4123		0.1021	0.5387	0.0568	0.5798

cause of the following reasons: firstly, the thinness of the body wall, which is a common feature of the genera *Ochetostoma* and *Thalassema*, facilitates diffusion of oxygen. Secondly, the large surface area is further increased by elongation of the trunk usually seen in specimens inside the burrows in laboratory aquaria. Thirdly, the presence of a large quantity of body fluid and haemoglobin-containing coelomic corpuscles continually agitated by peristaltic movements.

The oxygen uptake of the detached proboscis in 9 specimens averaged 17.2% (range: 7.6–25.4%) of the combined uptake of detached trunk and proboscis. The proboscis is therefore an accessory but not indispensable respiratory organ, since proboscis-less trunks survive indefinitely. Because of the extensibility of both trunk and proboscis it is difficult to compare their available respiratory surface. Due to the different degrees of hydration between body wall and proboscis, the average oxygen uptake of the trunk was twice that of the proboscis per gm. wet weight, although on the basis of dry weight the average uptake was approximately equal (Table II).

It is obviously an advantage to the proboscis in being able to respire independently of the trunk, since at ebb tide when the proboscis is fully extended foraging on the surface of the wet sand its actively moving distal tip is some 25 cm. away from the trunk that lies in the oxygen-depleted water inside the burrow. The presence of a certain amount of coelomic fluid and coelomic corpuscles inside the proboscis during full extension presumably increases its efficiency as a respiratory organ. This respiratory function explains the survival of the proboscis several days after its severance from the trunk.

An indirect evidence in support of the respiratory function for the proboscis is

the length and extensibility of the proboscis in the genus *Ochetostoma* and the presence of gill-like processes along the ventral margins in the proximal part of the proboscis in *O. arkati* (Prashad, 1935; Wesenberg-Lund, 1959) and *O. atlantidei* (Wesenberg-Lund, 1959). These processes, Wesenberg-Lund (1959) suggested, may function as a respiratory organ. The large oxygen uptake by the proboscis in *O. erythrogrammon* suggests respiratory function for the entire available external surface of the proboscis. It would be interesting to know whether the outer row of processes in *O. atlantidei* would disappear with full extension of the proboscis; the inner row in *O. atlantidei* and the processes in *O. arkati* may well be mere folds and presumably disappear with full extension, since in *O. erythrogrammon* similar folds or processes occur transiently along the ventral margin when the proboscis contracts but disappear with full extension.

Although a study of the relative importance of the different sites of oxygen uptake in *Urechis caupo* is lacking, available evidence, such as the presence of a long, large, inflatable hindgut and cloaca (Fisher and MacGinitie, 1928a; Fisher, 1946) and the occurrence of inhalations and exhalations through the anus (Fisher and MacGinitie, 1928b; Redfield and Florkin, 1931; Hall, 1931; Fisher, 1946), points to the importance of the hindgut as a respiratory organ. Redfield and Florkin (1931) observed antiperistalsis of *Urechis* hindgut, obtained 25–35 cc. of water at a single discharge during anal exhalation and found that this water contained less oxygen but more carbon dioxide than aquarium water outside the body. They believed that the thick body wall of *Urechis* must absorb a relatively small amount of oxygen in comparison with the hindgut. The relative importance of the sites of respiratory exchange thus differs between the echiuroids,

TABLE III

Rates of oxygen consumption of some annelids, echiuroids and a sipunculoid

Animal	Author	Oxygen consumption cc./gm./hr.
<i>Tubifex</i>	Brazda (1939)	0.2
<i>Schizobranchia insignis</i>	Dales (1961)	0.1920
<i>Ochetostoma erythrogrammon</i>	Present author	0.0692
<i>Bispira voluticornis</i>	Zoond (1931)	0.0573
<i>Sabella</i>	Wells (1952)	0.0488
<i>Lumbricus terrestris</i>	Johnson (1942)	0.045
<i>Myxicola</i>	Wells (1952)	0.0398
<i>Sipunculus nudus</i>	Cohnheim (from Krogh, 1916)	0.0313–0.0688
<i>Arenicola marina</i>	Borden (1931)	0.031
<i>Nereis virens</i>	Bosworth, O'Brien and Amberson (1936)	0.026
<i>Hirudo</i>	Heilbrunn (1952)	0.023
<i>Urechis caupo</i>	Hall (1931)	0.0198
<i>Glycera siphonostoma</i>	Montuori (from Krogh, 1916)	0.0146
<i>Chaetopterus pergamentaceus</i>	Bosworth, O'Brien and Amberson (1936)	0.0078

Urechis and *Ochetostoma*. Presumably, in *Urechis* the thick body wall prevents rapid diffusion of oxygen and the small size of the proboscis offers only a small respiratory surface. Apart from members of the genus *Urechis*, only *Nellobia cusoma* has a large hindgut and cloaca (Fisher, 1946), which may have a respiratory function. All other known echiuroids have a slender coiled hindgut and small cloaca, which are obviously not adapted for efficient respiratory function, but they have a thin and presumably respiratory body wall.

Krogh (1916) pointed out the difficulty of comparing the metabolism of different invertebrate animals and that a fair comparison could not be made on the basis of fresh weight because of the enormous differences in the composition of the various animals. Although dry weight offers a better basis for comparisons, the presence of varying amounts of reserve material, skeletal and other inactive tissues also renders this far from ideal (Krogh, 1916). When the oxygen consumption of *O. erythrogrammon* based on wet weight of fixed tissues (*i.e.* minus coelomic fluid and gut fluid) is compared with annelids, sipunculoids and *Urechis*, it occupies a position near the top in the descending series of rates shown in Table III.

SUMMARY

1. The oxygen consumption of *Ochetostoma erythrogrammon* averaged 0.0692 cc. per hour per gram of wet weight of fixed tissue.
2. After blockade of the anus the oxygen uptake did not diminish, indicating that the cloaca and hindgut have no significant respiratory function.
3. Both trunk and proboscis took up oxygen after separation, the latter consuming oxygen averaging 17% of the combined uptake of trunk and proboscis.
4. The relative importance of cloaca and hindgut, trunk, and proboscis as respiratory organs was discussed.
5. The oxygen consumption of *O. erythrogrammon* was compared with related animals.

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